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(54) Porous tissue scaffoldings for the repair or regeneration of tissue

(57) The present patent describes a three-dimensional interconnected open cell porous foams that have a gradient in composition and/or microstructure through one or more directions. These foams can be made from a blend of absorbable and biocompatible polymers that are formed into foams having a compositional gradient transitioning from predominately one polymeric material to predominately a second polymeric material. These gradient foams are particularly well suited to tissue engineering applications and can be designed to mimic tissue transition or interface zones.

EP 1 064 958 A1

tissue.

Summary of Invention

[0009] The present invention provides a biocompatible gradient foam that has a substantially continuous transition in at least one characteristic selected from the group consisting of composition, stiffness, flexibility, bioabsorption rate pore architecture and/or microstructure. This gradient foam can be made from a blend of absorbable polymers that form compositional gradient transitions from one polymeric material to a second polymeric material. In situations where a single chemical composition is sufficient for the application, the invention provides a biocompatible foam that may have microstructural variations in the structure across one or more dimensions that may mimic the anatomical features of the tissue (e.g. cartilage, skin, bone etc.).

[0010] The present invention further provides biocompatible foam having interconnecting pores and channels to facilitate the transport of nutrients and/or invasion of cells into the scaffold. These biocompatible foams are especially well adapted for facilitating the ingrowth of tissue as is described in Example 7.

[0011] In yet another embodiment of the present invention biocompatible foams having interconnecting pores formed from a composition containing in the range of from about 30 weight percent to about 99 weight £-caprolactone repeating units are disclosed. These biocompatible foams are especially well adapted for facilitating the growth of osteoblasts as is described in Example 6.

[0012] The present invention also provides a method for the repair or regeneration of tissue contacting a first tissue with a gradient foam at a location on the foam that has appropriate properties to facilitate the growth of said tissue. The concept of a continuous transition in physical properties, chemical composition and/or microstructural features in the porous scaffold (foam) can facilitate the growth or regeneration of tissue. These foam structures are particularly useful for the generation of tissue junctions between two or more different types of tissues. For a multi-cellular system in the simplest case, one cell type could be present on one side of the scaffold and a second cell type on the other side of the scaffold. Examples of such regeneration can be (a) skin: with fibroblasts on one side to regenerate dermis, and keratinocytes on the other to regenerate *epidermis;* (b) vascular grafts: with an endothelial layer on the inside of the graft and a smooth muscle cell layer on the outside.

Brief Description of Figures

[0013] Figure 1 is a scanning electron micrograph of the cross section of a random microstructure foam made from 5% solution of 35/65 ε-caprolactone-co-glycolide copolymer.

[0014] Figure 2 is a scanning electron micrograph of the cross section of a foam with vertical open channels made from 10% solution of 35/65 ε-caprolactone-co-glycolide copolymer.

[0015] Figure 3 is a scanning electron micrograph of the cross section of a foam with architectural gradient made from 10% solution of 35/65 ε-caprolactone-co-glycolide copolymer.

[0016] Figure 4 is a scanning electron micrograph of the cross section of a gradient foam made from a 50/50 blend of 40/60 ε-caprolactone-co-(L)lactide copolymer and 35/65 ε-caprolactone-co-glycolide copolymer.

[0017] Figure 5 is a scanning electron micrograph of a cross section of the top portion of a gradient foam made from a 50/50 blend of 40/60 ε-caprolactone-co-(L)lactide copolymer and 35/65 ε-caprolactone-co-glycolide copolymer.

[0018] Figure 6 is a scanning electron micrograph of a cross section of the bottom portion of a gradient foam made from a 50/50 blend of 40/60 ε-caprolactone-co-(L)lactide copolymer and 35/65 ε-caprolactone-co-glycolide copolymer.

[0019] Figure 7 is a graphical presentation of cell culture data, 7A, 7B and 7C.

[0020] Figure 8 is an anatomical sketch of cartilage tissue.

[0021] Figure 9A, 9B, and 9C are scanning electron micrographs of a 0.5 mm foam made from a 50/50 blend of a 35/65 ε-caprolactone-co-glycolide copolymer and a 40/60 ε-caprolactone-co-(L)lactide copolymer with architecture suitable for use as a skin scaffold. Figure 9A shows the porosity of the surface of the scaffold that preferably would face the wound bed. Figure 9B shows the porosity of the surface of the scaffolding that would preferably face away from the wound bed. Figure 9C shows a cross section of the scaffold with channels running through the thickness of the foam.

[0022] Figure 10 is a dark field 40X photomicrograph of a trichrome stained sample illustrating the cellular invasion of the foam shown in Figure 9, eight days after implantation in a swine model.

[0023] Figure 11 is a 100X composite photomicrograph of a trichrome stained sample illustrating the cellular invasion of the foam shown in Figure 9 which also contained PDGF, eight days after implantation in a swine model.

Detailed Description of the Invention

[0024] This invention describes porous bioabsorbable polymer foams that have novel microstructures. The features

is adjacent to the tide mark, and a transition zone between the bone and cartilage that is composed of calcified cartilage. Subchondral bone is located adjacent to the tide mark and this transitions into cancellous bone. In the superficial or tangential zone, the collagen fibrils are parallel to the surface. The fibers are oriented to resist shear forces generated during normal joint articulation. The middle zone has a randomly arranged organization of much larger diameter collagen fibers. Finally, in the deep zone there are larger collagen fiber bundles, which are perpendicular to the surface, and they insert into the calcified cartilage. The cells are speroidiol and tend to arrange themselves in a columnar manner. The calcified cartilage zone has smaller cells with relatively little cytoplasm.

[0030] A preferred embodiment of this invention would be to generate a gradient foam structure that could act as a template for multiple distinct zones. These foam structures could be fabricated in a variety of shapes to regenerate or repair osteochondrial defects and cartilage. One potential foam structure would be cylindrical in shape with an approximate dimensions of 10mm in diameter and 10 mm in depth. The top surface is would be approximately 1 mm thick and would be a low porosity layer to control the fluid permeability. By adopting a suitable processing method the surface porosity of the foam could be controlled. The porosity of this skin like surface can be varied from completely impervious to completely porous. Fluid permeability would be controlled by surface porosity. Below such a skin the structure would consist of three zones. An upper porous zone which lies adjacent to cartilage tissue, a lower porous zone which lies adjacent to bone tissue, and a transition zone between the upper and lower porous zones. For articular cartilage, it is currently preferred that the stiffness (modulus) of the upper and lower porous layers at the time of implantation be at least as stiff, as the corresponding adjacent tissue. In such a case the porous layers will be able to support the environmental loading and thereby protect the invading cells until they have differentiated and consolidated into tissue that is capable of sustaining load. For example the porous structure used for the superficial tangential zone could have elongated pores and the orientation of the structure could be parallel to the surface of the host cartilage. However, the deep zone may have a porosity of about 80 to about 95 % with pores that are of the order of 100 µm (about 80 µm to about 120 µm). It is expected that chondrocytes will invade this zone. Below this, would be a zone with larger pores (about 100 µm to about 200 µm) and a porosity in the range of about 50 to about 80 %. Such 100 µm to about 200 µm porous foam would have a structure such that the struts or walls of the pores are larger and vertical to the load, similar to the naturally occurring structure and to bear the loads. Finally, at the bottom of this structure there is a need for larger pores (about 150 µm to about 300 µm) with higher stiffness to be structurally compatible with cancellous bone. The foam in this section could be reinforced with ceramic particles or fibers made up of calcium phosphates and the like.

[0031] Recent data generated in our laboratories support the hypothesis that cell invasion can be controlled by pore size. In these studies, a scaffold made of 95/5 mole percent poly(L)lactide-co-ε-caprolactone) with an approximate pore size of about 80 µm had chondrocyte invasion of about 30 cells/mm^2 of the scaffold (under static conditions). Scaffolds made of 40/60 mole percent poly(ε-caprolactone-co-(L)lactide) with a larger approximate pore size of about 100 µm had a statistically significantly greater cellular invasion of 50 cells/mm^2(under static conditions). In both cases the cells were bovine chondrocytes. A very simple gradient structure with a variation of pore sized from about 80 µm to about 150 µm would provide a structure where chondrocytes would more easily invade the area with larger pores. The area with smaller pores would be void of chondrocytes or would be filled with a second cell types (e.g., fibroblasts).

[0032] In a compositionally gradient foam a blend of two or more elastomeric copolymers or in combination with high modulus semi-crystalline polymers along with additives such as growth factors or particulates can be chosen such that first a desired pore gradient is developed with a preferred spatial organization of the additives. Then using a variety of the approaches referred to in the preferred methods of making gradient foams, a compositional gradient can be superimposed primarily due to the differences in the polymer-solvent phase separation behavior of each system. Such a gradient foam structure would elicit a favorable response to chondrocytes or osteoblasts depending on the spatial location.

[0033] Further, the purpose of a functional gradient is to more evenly distribute the stresses across a region through which mechanical and/or physical properties are varying and thereby alleviate the stress concentrating effects of a sudden interface. This more closely resembles the actual biological tissues and structures, where structural transitions between differing tissues such as cartilage and bone are gradual. Therefore, it is an object of the present invention to provide an implant with a functional gradient between material phases. The present invention provides a multi-phasic functionally graded bioabsorbable implant with attachment means for use in surgical repair of osteochondral defects or sites of osteoarthritis. Several patents have proposed systems for repairing cartilage that could be used with the present inventive porous scaffolds. For example, U.S. Patent 5,769,899 describes a device for repairing cartilage defects and U.S. Patent 5,713,374 describes securing cartilage repair devices with bone anchors (both hereby incorporated herein by reference).

Bone

[0034] Gradient structures naturally occur for the bone / cartilage interface. In a study in our laboratories, we have demonstrated that material differences significantly influence cell function. In initial and long-term response of primary

foam tissue scaffolding shown in Figure 11. In compromised wound healing models the addition of a growth factor such as PDGF may in fact be necessary.

[0039] From our initial studies it appears that it is desirable to use as a skin scaffold a foam tissue scaffold having a thickness of from about 150 µm to about 3 mm, preferably the thickness of the foam may be in the range of from about 300 µm to about 1500 µm and most preferably about 500 to about 1000 µm. Clearly different skin injuries (i.e. diabetic ulcers, venous stasis ulcers, decubitis ulcers, burns etc.) may require different foam thickness. Additionally, the patient's condition may necessitate the incorporation of growth factors, antibiotics and antifungal compounds to facilitate wound healing.

Vascular grafts

[0040] The creation of tubular structures with gradients may also be of interest. In vascular grafts, having a tube with pores in the outer diameter which transitions to smaller pores on the inner surface or visa versa may be useful in the culturing of endothelial cells and smooth muscle cells for the tissue culturing of vessels.

[0041] Multilayered tubular structures allow the regeneration of tissue that mimics the mechanical and/or biological characteristics of blood vessels will have utility as a vascular grafts. Concentric layers, made from different compositions under different processing conditions could have tailored mechanical properties, bioabsorption properties, and tissue ingrowth rates. The inner most, or luminal layer would be optimized for endothelialization through control of the porosity of the surface and the possible addition of a surface treatment. The outermost, or adventitial layer of the vascular graft would be tailored to induce tissue ingrowth, again by optimizing the porosity (percent porosity, pore size, pore shape and pore size distribution) and by incorporating bioactive factors, pharmaceutical agents, or cells. There may or may not be a barrier layer with low porosity between these two porous layers to increase strength and decrease leakage.

Composition of foams

[0042] A variety of absorbable polymers can be used to make foams. Examples of suitable biocompatible, bioabsorbable polymers that could be used include polymers selected from the group consisting of aliphatic polyesters, poly(amino acids), copoly(ether-esters), polyalkylenes oxalates, polyamides, poly(iminocarbonates), polyorthoesters, polyoxaesters, polyamidoesters, polyoxaesters containing amine groups, poly(anhydrides), polyphosphazenes, biomolecules and blends thereof. For the purpose of this invention aliphatic polyesters include but are not limited to homopolymers and copolymers of lactide (which includes lactic acid, D-,L- and meso lactide), glycolide (including glycolic acid), ε-caprolactone, p-dioxanone (1,4-dioxan-2-one), trimethylene carbonate (1,3-dioxan-2-one), alkyl derivatives of trimethylene carbonate, δ-valerolactone, β-butyrolactone, γ-butyrolactone, ε-decalactone, hydroxybutyrate (repeating units), hydroxyvalerate (repeating units), 1,4-dioxepan-2-one (including its dimer 1,5,8,12-tetraoxacyclotetradecane-7,14-dione), 1,5-dioxepan-2-one, 6,6-dimethyl-1,4-dioxan-2-one 2,5-diketomorpholine, pivalolactone, alpha, alpha-diethylpropiolactone, ethylene carbonate, ethylene oxalate, 3-methyl-1,4-dioxane-2,5-dione, 3,3-diethyl-1,4-dioxan-2,5-dione, 6,8-dioxabicycloctane-7-one and polymer blends thereof. Poly(iminocarbonate) for the purpose of this invention include as described by Kemnitzer and Kohn, in the Handbook of Biodegradable Polymers, edited by Domb, Kost and Wisemen, Hardwood Academic Press, 1997, pages 251-272. Copoly(ether-esters) for the purpose of this invention include those copolyester-ethers described in "Journal of Biomaterials Research", Vol. 22, pages 993-1009, 1988 by Cohn and Younes and Cohn, Polymer Preprints (ACS Division of Polymer Chemistry) Vol. 30(1), page 498, 1989 (e.g. PEO/PLA). Polyalkylene oxalates for the purpose of this invention include Patent Nos. 4,208,511; 4,141,087; 4,130,639; 4,140,678; 4,105,034; and 4,205,399 (incorporated by reference herein). Polyphosphazenes, co-, ter- and higher order mixed monomer based polymers made from L-lactide, D,L-lactide, lactic acid, glycolide, glycolic acid, para-dioxanone, trimethylene carbonate and ε-caprolactone such as are described by Allcock in The Encyclopedia of Polymer Science, Vol. 13, pages 31-41, Wiley Intersciences, John Wiley & Sons, 1988 and by Vandorpe, Schacht, Dejardin and Lemmouchi in the Handbook of Biodegradable Polymers, edited by Domb, Kost and Wisemen, Hardwood Academic Press, 1997, pages 161-182 (which are hereby incorporated by reference herein). Polyanhydrides from diacids of the form $HOOC\text{-}C_6H_4\text{-}O\text{-}(CH_2)_m\text{-}O\text{-}C_6H_4\text{-}COOH$ where m is an integer in the range of from 2 to 8 and copolymers thereof with aliphatic alpha-omega diacids of up to 12 carbons. Polyoxaesters, polyoxaamides and polyoxaesters containing amines and/or amido groups are described in one or more of the following U.S. Patent Nos. 5,464,929; 5,595,751; 5,597,579; 5,607,687; 5,618,552; 5,620,698; 5,645,850; 5,648,088; 5,698,213; 5,700,583; and 5,859,150 (which are incorporated herein by reference). Polyorthoesters such as those described by Heller in Handbook of Biodegradable Polymers, edited by Domb, Kost and Wisemen, Hardwood Academic Press, 1997, pages 99-118 (hereby incorporated herein by reference).

[0043] Currently aliphatic polyesters are the absorbable polymers that are preferred for making gradient foams. Aliphatic polyesters can be homopolymers, copolymers (random, block, segmented, tappered blocks, graft, triblock, etc.) having a linear, branched or star structure. Preferred are linear copolymers. Suitable monomers for making aliphat-

in EP 464,163 B1), gas injection extrusion, gas injection molding or casting with an extractable material (i.e., salts, sugar or any other means known to those skilled in the art). Currently it is preferred to prepare bioabsorbable, biocompatible elastomeric foams by lyophilization. Suitable methods for lyophilizing elastomeric polymers to form foams is described in the Examples and in the copending patent application entitled, "Process for Manufacturing Biomedical Foams", assigned to Ethicon, Inc., docket number ETH-1352, filed June 30, 1999 hereby incorporated herein by reference herein.

[0050] The foams that are made in this invention are made by a polymer-solvent phase separation technique with modifications to the prior art that unexpectedly creates gradients in the foam structure. Generally, a polymer solution can be separated into two phases by any one of the four techniques: (a) thermally induced gelation/crystalization; (b) non-solvent induced separation of solvent and polymer phases; (c) chemically induced phase separation, and (d) thermally induced spinodal decomposition. The polymer solution is separated in a controlled manner into either two distinct phases or two bicontinuous phases. Subsequent removal of the solvent phase usually leaves a porous structure of density less than the bulk polymer and pores in the micrometer ranges (ref. "Microcellular foams via phase separation" by A. T. Young, J. Vac. Sci. Technolol. A 4(3), May/Jun 1986). The steps involved in the preparation of these foams consists of choosing the right solvents for the polymers that needs to be lyophilized and preparing a homogeneous solution. Next, the polymer solution is subjected to a freezing and vacuum drying cycle. The freezing step phase separates the polymer solution and vacuum drying step removes the solvent by sublimation and/or drying leaving a porous polymer structure or an interconnected open cell porous foam.

[0051] Suitable solvents that should be generally suited as a starting place for selecting a solvent for the preferred absorbable aliphatic polyesters include but are not limited to solvents selected from a group consisting of formic acid, ethyl formate, acetic acid, hexafluoroisopropanol (HFIP),cyclic ethers (i.e. THF, DMF, and PDO), acetone, acetates of C2 to C5 alcohol (such as ethyl acetate and t-butylacetate),glyme (i.e. monoglyme, ethyl glyme, diglyme, ethyl diglyme, triglyme, butyl diglyme and tetraglyme) methylethyl ketone, dipropyleneglycol methyl ether, lactones (such as γ-valerolactone, δ-valerolactone, β-butyrolactone, γ-butyrolactone) 1,4-dioxane, 1,3-dioxolane, 1,3-dioxolane-2-one (ethylene carbonato), dimethlycarbonate, benzene, toluene, benzyl alcohol, p-xylene, naphthalene, tetrahydrofuran, N-methyl pyrrolidone, dimethylformamide, chloroform, 1,2-dichloromethane, morpholine, dimethylsulfoxide, hexafluoroacetone sesquihydrate (HFAS), anisole and mixtures thereof. Among these solvents, the preferred solvent is 1,4-dioxane. A homogeneous solution of the polymer in the solvent is prepared using standard techniques.

[0052] Accordingly, as will be appreciated, the applicable polymer concentration or amount of solvent, which may be utilized, will vary with each system. Suitable phase diagram curves for several systems have already been developed. However, if an appropriate curve is not available, this can be readily developed by known techniques. For example, a suitable technique is set forth in Smolders, van Aartsen and Steenbergen, Kolloid--Z. u. Z. Polymere, 243, 14 (1971). As a general guideline the amount of polymer in the solution can vary from about 0.5% to about 90% and preferably will vary from about 0.5% to about 30% by weight depending to a large extent on the solubility of the polymer in a given solvent and the final properties of the foam desired.

[0053] Additionally, solids may be added to the polymer-solvent system. The solids added to the polymer-solvent system preferably will not react with the polymer or the solvent. Suitable solids include materials that promote tissue regeneration or regrowth, buffers, reinforcing materials or porosity modifiers. Suitable solids include, but are not limited to, particles of demineralized bone, calcium phosphate particles, or calcium carbonate particles for bone repair, leachable solids for pore creation and particles of bioabsorbable polymers not soluble in the solvent system as reinforcing or to create pores as they are absorbed. Suitable leachable solids include but are not limited nontoxic leachable materials selected from the group consisting of salts (i.e. sodium chloride, potassium chloride, calcium chloride, sodium tartrate, sodium citrate, and the like) biocompatible mono and disaccharides (i.e. glucose, fructose, dextrose, maltose, lactose and sucrose), polysaccharides (i.e. starch, alginate), water soluble proteins (i.e. gelatin and agarose). Generally all of these materials will have an average diameter of less than about 1mm and preferably will have an average diameter of from about 50 to about 500 μm. The particles will generally constitute from about 1 to about 50 volume percent of the total volume of the particle and polymer-solvent mixture (wherein the total volume percent equals 100 volume percent). The leachable materials can be removed by immersing the foam with the leachable material in a solvent in which the particle is soluble for a sufficient amount of time to allow leaching of substantially all of the particles, but which does not dissolve or detrimentally alter the foam. The preferred extraction solvent is water, most preferably distilled-deionized water. This process is described in U.S. patent No. 5,514,378 hereby incorporated herein by reference (see column 6). Preferably the foam will be dried after the leaching process is complete at low temperature and/or vacuum to minimize hydrolysis of the foam unless accelerated absorption of the foam is desired.

[0054] After the polymer solvent mixture is formed the mixture is then solidified. For a specific polymer-solvent system, the solidification point, the melt temperature and the apparent glass transition of the polymer-solvent system can be determined using standard differential scanning calorimetric (DSC) techniques. In theory, but in no way limiting the scope of the present invention, it is believed that as a polymer solvent system is cooled down an initial solidification occurs at about or below the freezing point of the solvent. This corresponds to the freezing of a substantial portion of

diately after the solution apparently solidifies. However, if the same solution is allowed to solidify further the foam will have larger pores closer to the surface where the vacuum is being drawn (opposite the heat sink) and smaller pores closer to the heat sink.

[0062] This process is the antitheses of the prior art process that focused on creating foams with a uniform microstructure (randomly interconnected pores), whereby the whole solution is completely frozen. And vacuum drying is applied only after a considerable amount of time is given for the completion of desired phase separation (U.S. Patents 5,755,792 (Brekke); 5,133,755 (Brekke); 5,716,413 (Walter, et al.); 5,607,474 (Athanasiou, et al.); 5,686,091 (Leong, et al.); 5,677,355 (Shalaby, et al.); and European disclosures E0274898 (Hinsch) and EPA 594148 (Totakura)).

[0063] Foams with various structures are shown in Figures 2, 3, and 4. For example, as shown in Figure 3 the orientation of the major axis of the pores may be changed from being in the same plane as the foam to being oriented perpendicular to the plane of the foam. By way of theory, but in no way limiting the scope of this invention, it is believed that this in conventional foam processing as the solvent crystallizes a freezing front moves through the solution solidifying the solution in crystalline layers parallel to the freezing front. However, if a vacuum is pulled before the solution completely freezes, the morphology of the foam results in pores being formed generally aligned parallel to the vacuum source. As is illustrated in Figure 3.

[0064] As can be seen from Figure 3 the pore size can be varied from a small pore size generally between about 10 µm and about 60 µm to a larger size of from about 60 µm to about 200 µm in a porous gradient foam. Again this results from pulling a vacuum on the apparently solidified solution before it is completely solidified. The polymer concentration in the solution and the cooling rates are also important parameters in controlling the cell size. Ideally the foam structure could be created to serve as a template to restore human tissue junctions such as the cartilage to bone junction present in joints. This foam would progress form a small round pores to larger column-like (i.e. with a diameter to length ratio of at least 2 to 1) pores. Additionally, the stiffness of the foam can controlled by the foams structure or blending two different polymers with different Young's moduli.

[0065] Foams can also have channels as is illustrated in Figure 2. The channels formed by this process may traverse the thickness of the foam and generally range in diameter from about 30 to about 200 µm in diameter. The channels generally are at least two times the channel's average diameter and preferably are at least four times the channel's average diameter and most preferably at least eight times the channel's average diameter. The channel size and diameter of course will be selected based on the desired functionality of the channel such as cell invasion, nutrient diffusion or as a avenue for vascularization.

[0066] One skilled in the art can easily visualize that such a directionality can be created in any three dimensions by designing an appropriate mold and subjecting the walls of such a mold to different temperatures if needed. The following types of gradient structures can be made by variation in the pore size and/or shape through the thickness with a uniform composition: pores of one type and size for a certain thickness followed by another type and size of pores, etc; compositional gradient with predominantly one composition on one side and another one on the other with a transition from one entity to the other; a thick skin comprising low porosity of low pore size layer followed by a large pore size region; foams with vertical pores with a spatial organization these vertical pores can act as channels for nutrient diffusion the making of these in 3D molds to create 3D foams with the desired microstructure combinations of compositional and architectural gradient. Generally the foams formed in containers or molds will have a thickness in the range of from about 0.25mm to about 100mm and preferably will have a thickness of from about 0.5mm to about 50mm. Thicker foams can be made but the lyophilization cycle times may be quite long, the final foam structures may be more difficult to control and the residual solvent content may be higher.

[0067] As previously described the inventive process cycle for producing biocompatible foam is significantly reduced by performing the sublimation step above the apparent glass transition temperature and below the solidification temperature of the mixture (preferably just below the solidification temperature). The combined cycle time of (freezing + primary drying + secondary drying) is much faster than is described in the prior art. For example, the combined cycle for aliphatic polyesters using volatile solvents is generally less than 72 hours, preferably less than 48 hours, more preferably less than 24 hours and most preferably less than 10 hours. In fact the combined cycle can be performed with some aliphatic polyesters in less than 3 hrs for foams of thickness 1mm or less; less than 6 hrs for foams of thickness around 2 mm and less than 9 hrs for foams of thickness around 3 mm. Compare this with prior art which is typically 72 hours or greater. The residual solvent concentrations in these foams made by this process will be very low. As described for aliphatic polyesters foams made using 1,4-dioxane as a solvent the residual concentration of 1,4-dioxane was less than 10 ppm (parts per million) more preferably less than 1 ppm and most preferably less than 100 ppb (parts per billion).

[0068] One skilled in the art can easily visualize that such a directionality can be created in any three-dimensions by designing an appropriate mold and subjecting the walls of such a mold to different temperatures if needed. The following types of gradient structures can be made by this invention

1. variation in the pore size and/or shape through the thickness with a uniform composition,

a specific cell type (i.e., pluripotent cells, stem cells or precursor cells such as the mesenchymal stem cells described in Caplan, U.S. 5,486,359, etc.). Suitable cell that may be contacted or seeded into the foam scaffolds include but are not limited to myocytes, adipocytes, fibromyoblasts, ectodermal cell, muscle cells, osteoblast (i.e. bone cells), chondrocyte (i.e. cartilage cells), endothelial cells, fibroblast, pancreatic cells, hepatocyte, bile duct cells, bone marrow cells, neural cells, genitourinary cells (including nephritic cells) and combinations thereof. Various cellular strategies could be used with these scaffolds (i.e., autogenous, allogenic, xenogeneic cells etc.). The cells could also contain inserted DNA encoding a protein that could stimulate the attachment, proliferation or differentiation of tissue. The foam would be placed in cell culture and the cells seeded onto or into the structure. The foam would be maintained in a sterile environment and then implanted into the donor patient once the cells have invaded the microstructure of the device. The in vitro seeding of cells could provide for a more rapid development and differentiation process for the tissue. It is clear that cellular differentiation and the creation of tissue specific extracellular matrix is critical for the tissue engineering of a functional implant.

[0076] The option for seeding different cell types into the different pore structures would be available to investigators. Schaufer et al., have demonstrated that different cell types (i.e. stromal cells and chondrocytes) can be cultured on different structures. The structures can be combined after a short period of time and the entire structure can be placed back in cell culture so a biphasic tissue structure can be generated for implantation. A gradient structure would also allow for co-cultured tissue scaffolds to be generated. (Schaefer, D. et al.). Additionally, radio-opaque markers may be added to the foams to allow imaging after implantation. After a defined period of in vitro development (for example 3 weeks), the tissue engineered implant would be harvested and implanted into the patient. If an acellular strategy is pursued, then the sterile acellular scaffolds would be used to replace damaged or traumatized tissue.

[0077] The foam scaffolds of the present invention may be sterilized using conventional sterilization process such as radiation based sterilization (i.e. gamma-ray), chemical based sterilization (ethylene oxide) or other appropriate procedures. Preferably the sterilization process will be with ethylene oxide at a temperature between 52-55°C for a time of 8 hours or less. After sterilization the foam scaffolds may be packaged in an appropriate sterilize moisture resistant package for shipment and use in hospitals and other health care facilities.

[0078] The following examples are illustrative of the principles and practice of this invention, although not limited thereto. Numerous additional embodiments within the scope and spirit of the invention will become apparent to those skilled in the art.

Examples

[0079] In the examples which follow, the polymers and monomers were characterized for chemical composition and purity (NMR, FT-IR), thermal analysis (DSC), molecular weight (inherent viscosity), and baseline and in vitro mechanical properties (Instron stress/strain).

[0080] ^1H NMR was performed on a 300 MHz NMR using $CDCl_3$ or HFAD (hexafluoroacetone sesqua deutrium oxide) as a solvent. Thermal analysis of segmented polymers and monomers was performed on a Dupont 912 Differential Scanning Calorimeter (DSC). Inherent viscosities (I.V., dL/g) of the polymers and copolymers were measured using a 50 bore Cannon-Ubbelhode dilution viscometer immersed in a thermostatically controlled water bath at 25°C utilizing chloroform or hexafluoroisopropanol (HFIP) as the solvent at a concentration of 0.1 g/dL.

[0081] In these examples certain abbreviations are usde such as PCL to indicate polymerized ε-caprolactone, PGA to indicate polymerized glycolide, PLA to indicate polymerized (L)lactide. Additionally, the percentages in front of the copolymer indicates the respective mole percentages of each constituent.

Example 1

Preparation of a foam with random microstructure (no preferred architecture)

Step A. Preparing 5% wt./wt. homogeneous solution of 35/65 PCL/PGA in 1,4-Dioxane

[0082] A 5 % wt./wt. polymer solution is prepared by dissolving 1 part of 35/65 PCL/PGA with 19 parts of the solvent - 1,4-dioxane. The 35/65 PCL/PGA copolymer was made substantially as described in Example 8. The solution is prepared in a flask with a magnetic stir bar. For the copolymer to dissolve completely, it is recommended that the mixture is gently heated to 60 ± 5°C and continuously stirred for a minimum of 4 hours but not exceeding 8 hours. A clear homogeneous solution is then obtained by filtering the solution through an extra coarse porosity filter (Pyrex brand extraction thimble with fritted disc) using dry nitrogen to help in the filtration of this viscous solution.

with nitrogen. The chamber was purged with dry nitrogen for approximately 30 minutes before opening the door.

[0087] Figure 2 is a SEM picture that shows a cross section of the foam with vertical channels. These channels run through the thickness of the foam.

Example 3

Architecturally gradient foam

[0088] This example describes the making of a foam that has a gradient in foam morphology as shown in Figure 3 using a 10% solution of 35/65 ε-caprolactone-co-glycolide. The method used to make such a foam is similar to the description given in Example 2 with one difference. In step (ii) of the lyophilization process the time for which the solution is kept at the freezing step is 30 minutes.

[0089] Figure 3 is a scanning electron micrograph of a cross section of this foam. Note the variation in the pore size and pore shape through the thickness of the foam.

Example 4

Transcompositional foam

[0090] This example describes the making of a foam that has a compositional gradient and not necessarily a morphological gradient. Such a foam is made from polymer solutions that have been made from physical mixtures of two or more polymers. This example describes a transcompositional foam made from 35/65 PCL/PGA and 40/60 PCL/PLA

Step A. Preparing a solution mixture of 35/65 PCL/PGA and 40/60 PCL/PLA in 1,4-Dioxane

[0091] In the preferred method the two separate solutions are first prepared (a) a 10% wt/wt polymer solution of 35/65 PCL/PGA and (b) a 10% wt/wt 40/60 PCL/PLA. Once these solutions are prepared as described in Example 1, equal parts of each solution was poured into one mixing flask. The polymers used to make these solutions are described in Examples 8 and 9. A homogeneous solution of this physical mixture was obtained by gently heating to 60 ± 5°C and continuously stirring using a magnetic stir bar for approximately 2 hours.

Step B. Lyophilization cycle

[0092] We used an FTS Dura Dry Freeze dryer with computer control and data monitoring system to make this foam. The first step in the preparation of such a foam was to generate a homogeneous solution as described in Step A. The solution was carefully filled into a dish just before the actual start of the cycle. The cylindrical glass dish weighed 117 grams, was optical glass 2.5mm thick and cylindrical with a 100mm outer diameter and a 95mm inner diameter. The lip height of the dish was 50mm. Next the following steps were followed in sequence to make a 25mm thick foam with the transcompositional gradient:

(i). The solution filled dish was placed on the freeze dryer shelf and the solution conditioned at 20°C for 30 minutes. The cycle was started and the shelf temperature was set to -5°C with a programmed cooling rate of 0.5°C/min.

(ii). The solution was held at the freezing condition (-5°C) for 5 hours.

(iii). Vacuum was applied to initiate primary drying of the dioxane by sublimation and held at 100 milliTorr for 5 hours.

(iv): Next, secondary drying was done at 5°C for 5 hours and at 20°C for 10 hours. At each temperature the vacuum level was maintained at 20 mTorr.

(v). At the end of the second stage, the lyophilizer was brought to room temperature and the vacuum was broken with nitrogen. The chamber was purged with dry nitrogen for approximately 30 minutes before opening the door.

[0093] The foam has a gradient in chemical composition which is evident from a close scrutiny of the foam wall morphology as shown in Figure 4, 5 and 6. The gradient in the chemical composition was further supported by NMR data as detailed below:

[0094] Foam sample produced by the above method and which was approximately 25 mm thick was characterized

Differentiation assays:

[0100] Alkaline phosphatase activity: Alkaline phosphatase activity was determined by a colorimetric assay using *p*-nitrophenol phosphate substrate (Sigma 104) and following manufacturers instruction. Briefly, cells were seeded on the films or meshes at a density of 40,000 cells/well and incubated for 1, 6. 14, and 21 d. Once cells reached confluence at day 6 they were fed with mineralization medium (growth medium supplemented with 10mM β-glycerophosphate, 50 µg/ml ascorbic acid). Alkaline phosphatase activity was determined in cell homogenates (0.05% Triton X-100) at the above time points. The quantity of protein in cell extracts was determined by micro BCA reagent from Pierce. Primary rat osteoblasts cultured on films and meshes were also stained for membrane-bound alkaline phosphatase using a histochemical staining kit (Sigma). For all the films and meshes three samples per group were tested.

[0101] Osteocalin ELISA: Osteocalcin secreted into the medium by osteoblasts cultured on various films was quantified by ELISA (Osteocalcin ELISA kit, Biomedical Technologies Inc, Boston). Aliquots of media from the wells containing the polymer films were lyophilized prior to measurements of this protein by ELISA. Three samples for each polymer were tested and the ELISA was repeated twice.

Von Kossa staining

[0102] Three samples for each polymer were stained for mineralized tissue using Von Kossa silver nitrate staining.

Expression of alkaline phosphatase and osteocalcin mRNAs

[0103] The expression of alkaline phosphatase and osteocalcin mRNAs in cells was assessed by semi-quantitative reverse transcriptase polymerase chain reaction (RT-PCR) using RNA extracted from cells cultured for 21 d on the films. Seven days after seeding, the culture media was replaced with mineralization media (3 mM β-glycerophosphate and 50 µg/ml of ascorbic acid were added). The cells were cultured for additional 2 weeks, for a total period of 3 weeks. Total RNA was extracted from four samples per group using a RNeasy mini kit provided by Qiagen. The quality and amount of total RNA was measured for each polymer group. Total RNA was reverse transcribed to obtain cDNA using a reverse transcriptase reaction (Superscript II, Gibco). The cDNAs for osteocalcin, alkaline phosphatase, and Glyceraldehyde-3-phosphate-dehydrogenase (GAPDH) were amplified using a PCR protocol described previously (GIBCO BRL manufacturers instruction). The primer sequences (Table I) for osteocalcin, alkaline phosphatase, and GAPDH were obtained using the FASTA program (Genetic Computer Group, Madison, WI). Preliminary studies were also conducted to optimize the number of PCR cycles for each primer (Table II), and to determine the range of RNA, which exhibits proportionality to cDNA. The PCR products were electrophoreses on 1% (wt) agarose gels containing ethidium bromide. The gels were photographed under UV light and were evaluated by densitometry for the expression of osteocalcin and alkaline phosphatase mRNAs relative to GAPDH.

Statistical Anlysis

[0104] Analysis of variance (ANOVA) with Tukey *post* hoc comparisons was used to assess levels of significance for all the assays.

Table I.

Primers used in RT-PCR

Gene	Species	Forward primer	Reverse primer	Size (bp)
Alkaline phosphatase	Rat	5' ATCGCCTATCAGCTAATGCAC	5' GCAAGAAGAAGCCTTTGGG	379
Osteocalcin	Rat/Human	5'CAACCCCAATTGTGACGAGC	5' TGGTGCGATCCATCACAGAG	339
GAPDH	Mouse/ Human/ Rat	5'ACCACAGTCCATGCCATCAC	5'TCCACCACCCTGTTGCTGTA	452

wound excisional model with and without PDGF being provided. The resulting four different samples were then evaluated.

[0111] A blinded histologic evaluation was performed on 48 full thickness excisional wounds from four pigs (12 sites per animal) explanted at 8 days following wounding. The assessment was performed on H&E stained slides. During the histologic assessment, the following parameters were ranked/evaluated across the specimen set 1) cellular invasion of the matrix-qualitative and quantitative assessments 2) infiltration of polymorphonuclear leukocytes (PMNs) into the contact zone (ventral surface) of the matrix, 3) inflammation in the granulation tissue bed below (ventral to) the matrix, 4) reaction of the epidermis to the matrix, and 5) degree of fragmentation of the matrix.

Animal Husbandry:

[0112] The pigs were housed individually in cages (with a minimum floor area of 10-sq. ft.) and given identification. All pigs were assigned an individual animal number. A tag was placed on each individual animal cage listing the animal number, species/strain, surgical date, surgical technique and duration of the experiment and date of euthanasia. Each animal was clearly marked with an animal number on the base of the neck using a permanent marker.

[0113] The animal rooms were maintained at the range of 40 to 70% R.H. and 15 to 24°C (59.0 to 75.2°F). The animals were fed with a standard pig chow once per day, but were fasted overnight prior to any experimental procedure requiring anesthesia. Water was available *ad libitum*. A daily light:dark cycle of 12:12 hours was adopted.

Anesthesia:

[0114] On the initial day of the study, days of evaluation and the day of necropsy, the animals were restrained and anesthetized with either an intramuscular injection of Tiletamine HCl plus Zolazepam HCl (Telazol®, Fort Dodge Animal Health, Fort Dodge, Iowa 4 mg/ml) and Xylazine (Rompun®, Bayer Corporation, Agriculture Division, Animal Health, Shawnee Mission, Kansas, 4mg/ml) or Isoflurane (AErrane® Fort Dodge Animal Health, Fort Dodge, Iowa) inhalatory anesthesia (5% vol.) administered via a nose cone. When the animal was in the surgical suite, it was maintained on Isoflurane (AErrane®) inhalatory anesthesia (2% vol.) administered via a nose cone. Food was available after recovery from each procedure.

Preparation of the Surgical Site:

[0115] One day prior to the surgical procedure, body weights were measured and the dorsal region of four pigs were clipped with an electric clipper equipped with a #40 surgical shaving blade. The shaved skin was then re-shaved closely with shaving cream and a razor and then rinsed. The shaved skin and entire animal (excluding the head) was then scrubbed with a surgical scrub brush-sponge with PCMX cleansing solution (Pharmaseal® Scrub Care® Baxter Healthcare Corporation, Pharmaseal Division, Valencia, California) and then with HIBICLENS® chlorhexidine gluconate (available from COE Laboratories, Incorporated, Chicago, Illinois). The animal was wiped dry with a sterile towel. Sterile NU-GAUZE* gauze (from Johnson & Johnson Medical Incorporated, Arlington, Texas) was placed over the dorsal surface of each animal and secured with WATERPROOF* tape (available from Johnson & Johnson Medical Incorporated, Arlington, Texas). The entire torso region of the animal was then wrapped with Spandage™ elastic stretch bandage (available from Medi-Tech International Corporation, Brooklyn, New York) to maintain a clean surface overnight.

[0116] On the day of surgery, immediately prior to delivering the animal to the surgical suite, the dorsal skin was again scrubbed using a surgical scrub brush-sponge with PCMX cleansing solution (Pharmaseal® Scrub Care®), rinsed and wiped dry using a sterile towel, as performed on the previous day. The animals were placed prone on the surgical table and wiped with 70% alcohol and dried with sterile gauze. Using a sterile surgical marker (availabe from Codman® a division of Johnson & Johnson Professional Incorporated, Raynham, Massachusetts) and an acetate template, marks were made on the dorsal skin according to the desired placement of each full-thickness wound.

Surgical Procedure:

[0117] Following anesthesia, under sterile conditions, twelve (12) full-thickness excisions (1.5 x 1.5 cm) per animal were made in two rows parallel to the spinal column on the left and right dorsal regions using a scalpel blade. A pair of scissors and/or scalpel blade was used to aid in the removal of skin and subcutaneous tissue. Bleeding was controlled by use of a sponge tamponade. Sufficient space was left between wounds to avoid wound-to-wound interference. The excised tissue was measured for thickness using a digital caliper.

buffered formalin. The tissue was prepared for frozen sections. The tissue was trimmed and mounted onto the object holder with Tissue-Tek® OCT Compound (sold by Sakura Finetechnical Company, Limited, Tokyo, Japan) and quickly frozen. The specimens were sectioned on the cryostat at 10 µm and stained with a frozen H&E stain.

HISTOLOGICAL ASSESSMENTS (Day 8 post-wounding):

[0126] Histological evaluations for granulation tissue (area and length) and epithelialization were assessed using H&E stained specimens using a magnification of 20-40X. Granulation tissue height was determined by dividing the area by the length.

[0127] Histopathological evaluation of the tissue samples was assessed using the H&E stained specimens, they were first assessed under 100x to 400x magnification.

RESULTS

[0128] There was cellular invasion into the interstices of the matrix in the majority of all test sites. In the majority of sites this invasion was true tissue ingrowth comprised of varying populations of fibroblasts, macrophages, macrophage giant cells, and endothelial-like cells, there also appeared to be capillary formation. Significant formation of dense fibrous connective tissue layer dorsal to the matrices essentially embedding the matrices in the tissue, was seen at several sites for the 0.5mm foams with and without PDGF. The 1mm matrices were either at the surface of the tissue bed or sloughed. Macrophage giant cell formation seemed to be greater in the 0.5mm versus the 1mm foam scaffolds. In sites where the 1mm foam was being sloughed or partially separated from the underlying granulation tissue there was death of the invading cells forming masses of pyknotic cell debris.

[0129] Complete incorporation of the matrix into the granulation tissue bed was seen at several sites for the 0.5mm foam scaffoldings. Figures 10 and 11 illustrate the incorporation of these matrices into the granulation tissue bed. Figure 10 is a dark filed 40X pictomicrograph of a trichrome stained tissue sample.

[0130] Figure 11 is a 100X composite photomicrograph of a trichrome stained sample illustrating the cellular invasion of a foam containing PDGF. Complete incorporation of the matrices into the granulation tissue bed is evident in both pictures. The dense fibrous tissue above the foam scaffolding is evident in both pictures. These results indicate the 0.5mm foams will provide a suitable substrate for the growth of epidermal tissue.

Example 8

Synthesis of a Random Poly(ε-caprolactone-co-glycolide)

[0131] A random copolymer of ε-caprolactone-glycolide with a 35/65 molar composition was synthesized by ring opening polymerization reaction. The method of synthesis was essentially the method described in U.S. Patent 5,468,253 in Example 6 (which is hereby incorporated herein by reference). The amount of diethylene glycol initiator added was adjusted to 1.15 mmole/mole of monomer to obtain the following characteristics of the dried polymer: The inherent viscosity (I.V.) of the copolymer was 1.59 dL/g in hexafluoroisopropanol at 25°C. The molar ratio of PCL/PGA was found to be 35.5/64.5 by proton NMR with about 0.5% residual monomer. The glass transition (Tg) and the melting points (Tm) of the copolymer were found to be -1°C, 60°C and 126°C respectively, by DSC.

Example 9

Synthesis of 40:60 Poly(ε-caprolactone-co-L-lactide) by Sequential Addition

[0132] In the glove box, 100 µL (33 µmol) of a 0.33 M stannous octoate solution in toluene, 115 µL (1.2 mmol) of diethylene glycol, 24.6 grams (170 mmol) of L-lactide, and 45.7 grams (400 mmol) of ε-caprolactone were transferred into a silanized, flame dried, two neck, 250 mL round bottom flask equipped with a stainless steel mechanical stirrer and a nitrogen gas blanket. The reaction flask was placed in an oil bath already set at 190°C and held there. Meanwhile, in the glove box, 62.0 grams (430 mmol) L-lactide were transferred into a flame dried, pressure equalizing addition funnel. The funnel was wrapped with heat tape and attached to the second neck of the reaction flask. After 6 hours at 190°C, the molten L-lactide was added to the reaction flask over 5 minutes. The reaction was continued overnight for a total reaction time of 24 hours at 190°C. The reaction was allowed to cool to room temperature overnight. The copolymer was isolated from the reaction flask by freezing in liquid nitrogen and breaking the glass. Any remaining glass fragments were removed from the copolymer using a bench grinder. The copolymer was again frozen with liquid nitrogen and broken off the mechanical stirring paddle. The copolymer was ground into a tared glass jar using a Wiley Mill and allowed to warm to room temperature in a vacuum oven overnight. 103.13 grams of 40:60 poly(ε-caprolactone-

rate and pore architecture from the first location to the second location of said biocompatible foam.

11. The foam of claim 9 or claim 10 wherein the interconnecting pores have a pore size in the range from 10 µm to 200 µm.

12. The foam of any one of claims 9 to 11 wherein the biocompatible foam has a porosity of in the range of from 20 to 98 percent.

13. The foam of any one of claims 9 to 12 wherein the biocompatible foam has channels.

14. The foam of claim 13 wherein the channels have an average length of at least 200 µm.

15. The foam of any one of claims 1 to 14 which is formed with an insert within the biocompatible gradient foam.

16. The foam of any one of claims 1 to 15 for use in the repair or regeneration of tissue.

FIG. 2

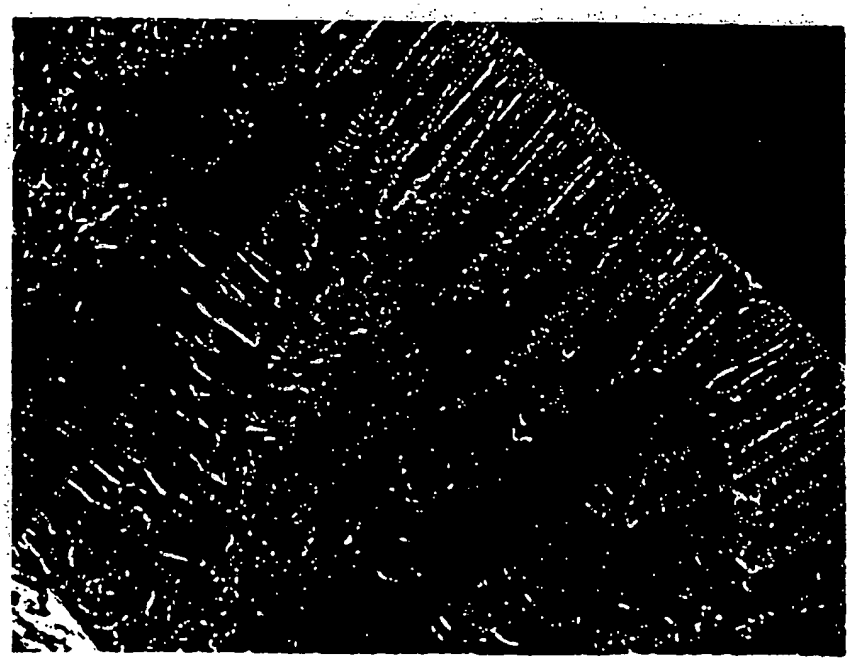

FIG. 4

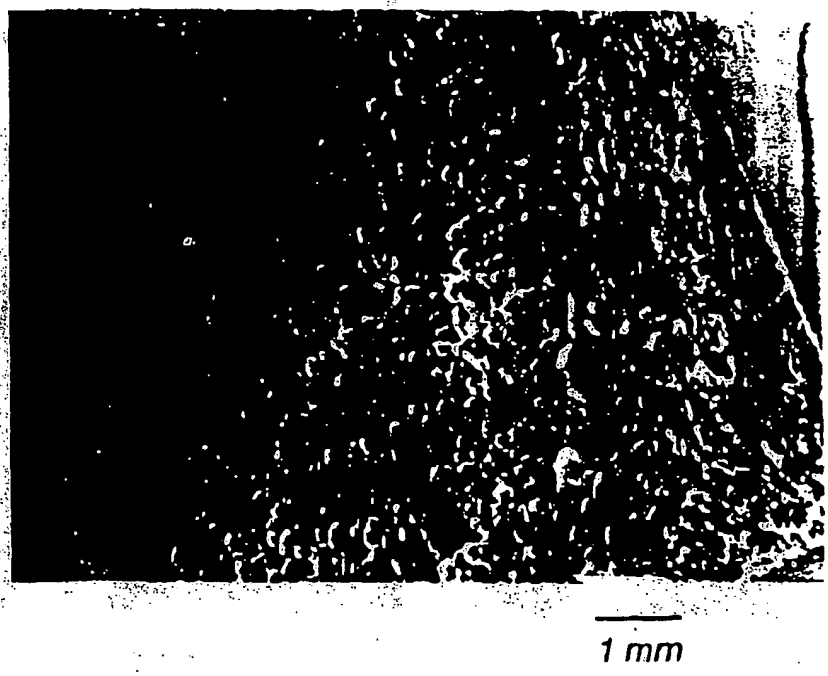

FIG. 6

FIG. 7B

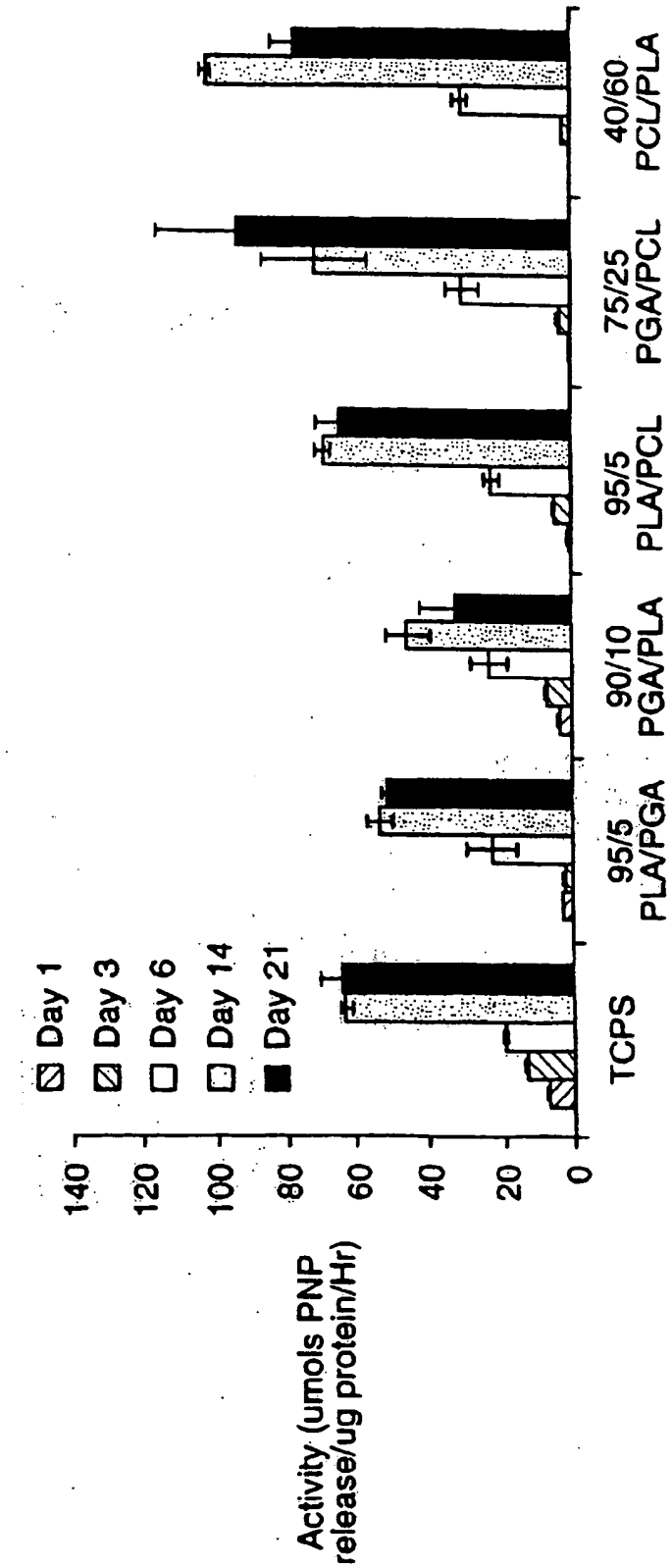
Alkaline Phosphatase Specific Activity on Bioresorbable films
Day 1
Day 3
Day 6
Day 14
Day 21
Activity (umols PNP release/ug protein/Hr)
0
20
40
60
80
100
120
140
TCPS
95/5 PLA/PGA
90/10 PGA/PLA
95/5 PLA/PCL
75/25 PGA/PCL
40/60 PCL/PLA

FIG. 8

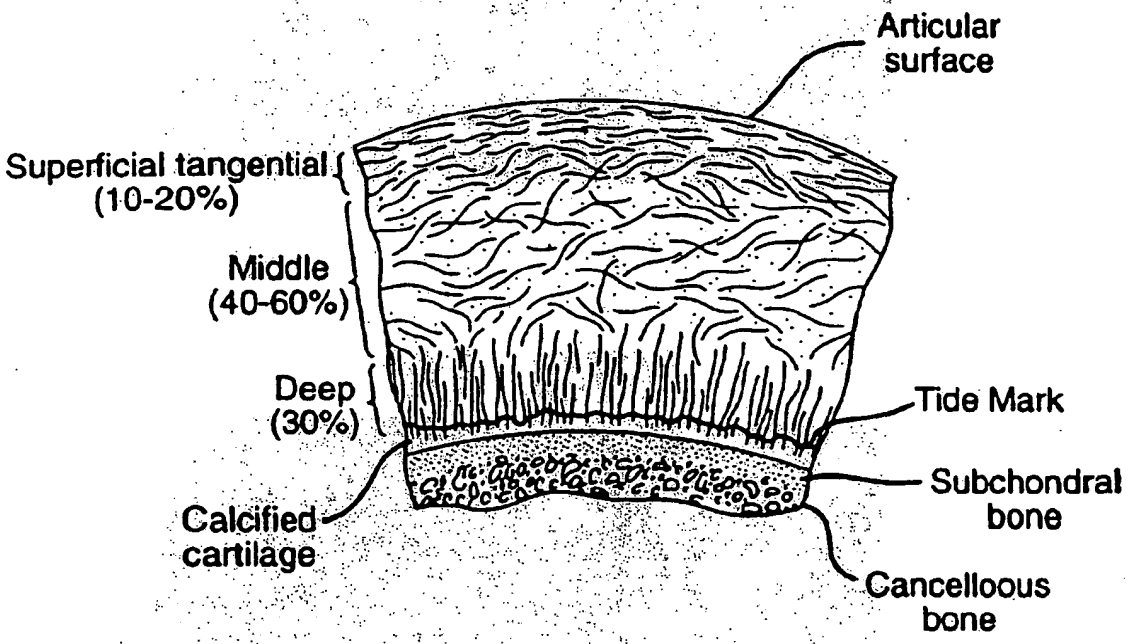

FIG. 9C

FIG. 11

ANNEX TO THE EUROPEAN SEARCH REPORT ON EUROPEAN PATENT APPLICATION NO. EP 00 30 5501

This annex lists the patent family members relating to the patent documents cited in the above-mentioned European search report. The members are as contained in the European Patent Office EDP file on
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20-10-2000

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